That response was deemed incomplete either because of the form of applicants' response or because additional figures were deemed to contain excessive text as well.

Applicants apologize for any misunderstanding at this stage, and enclose a set of substitute drawings (Figures 1 - 7) and a substitute Brief Description of the Drawings. Text from the drawings has been deleted and replaced with additional text in the Brief description of the Drawings. No new matter has been added.

If anything else is needed from applicants in order to move this case along in prosecution, applicants invite the Customer Service Center to call the undersigned attorney so that we can try to expedite resolution.

Respectfully submitted,

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I hereby certify that this paper is being deposited with the United States Postal Service as First Class Mail with sufficient postage, on the date indicated below and is addressed to the Commissioner for Patents, Washington, DC 20231.

Signed Sue Wilson Date: **May 10, 2002**
Sue Wilson

Brief Description of the Drawings

Fig 1 depicts the identification of receptor binding compounds and their use in the design of dimerizers. Step 1 illustrates the screening of natural product and diversity libraries to select a compound that binds to a receptor protein's extracellular domain. Step 2 illustrates the creation of a small molecule receptor agonist comprising two receptor binding molecules chemically linked together.

Fig 2 depicts the use of the dimerizer, FK1012, to trigger signaling in cells expressing an FKBP-CD3 zeta chain fusion protein. The figure illustrates the use of a divalent ligand to trigger cellular signaling leading in this case to the expression of IL-2 or another gene under the transcriptional control of the IL-2 promoter.

Fig 3 depicts the use of a dimerizer binding to the extracellular portion of a protein to trigger intracellular signaling. The figure illustrated the stimulation of an erythropoietin receptor by a small molecule ligand leading to activation of intracellular processes.

Fig 4 depicts the use of a competitive binding assay to identify compounds which bind to a receptor protein. The figure illustrates the identification of compounds which bind to a receptor protein using a competitive ligand binding assay. In well A of a 96-well microtiter plate no competitor has been added; in well B a non-competitive compound (dark circles) is also present; in well C a competitive compound (dark triangles) is present and is shown binding to the protein. The bar graph illustrating % maximal ligand binding per well illustrates the competitive binding depicted in well C.

Fig 5 depicts a screening assay to identify immobilized compounds which bind to a receptor protein. The figure illustrates the screening of molecular diversity libraries for compounds that bind to a receptor extracellular domain. The top panel depicts the incubation of fluorescently tagged protein molecules comprising a receptor extracellular domain with a collection of compounds synthesized on beads. The middle panel illustrates the removal of unbound receptor proteins and the visualization of fluorescence linked to bound beads. The bottom panel illustrates the recovery

of a thus visualized bead and the identification of the linked compound to which the protein had bound.

Fig 6 depicts EPO-induced signaling in cells expressing chimeric receptor proteins and the use of such systems to identify small molecule antagonists of EPO-binding. The top panel illustrates fusion proteins containing an erythropoietin receptor extracellular domain (1) and a T cell receptor zeta subunit intracellular domain (2), together with an erythropoietin molecule which binds to the hybrid receptor proteins and induces signal transduction leading to IL-2 production. The bottom panel illustrates the ability of a small molecule to block such EPO receptor-mediated signal transduction through binding of the small molecule to the receptor protein in place of erythropoietin, thus blocking IL-2 production.

Fig 7 depicts a general methodology for the design and construction of an expression vector for producing a portion of a receptor protein, *e.g.*, for use in binding experiments. The receptor binding domain can be identified by inspection of the receptor coding sequence (e.g. Kyte Doolittle analysis) or by analysis of deletion mutants (see Watowich et al, Mol. Cell. Biol. 14:3535 1994). PCR primes flanking the ligand binding domain (LBD) are used to PCR amplify the region encoding the LBD. By inclusion of sequences encoding a particular epitope in the other PCT primer, an epitope can be fused to the N- or C-terminus of the LBD. Other PCT primers can be used to introduce restriction sites into the ends of the LBD coding sequence to facilitate cloning. The cloned LBD is then ligated into an appropriate expression vector, such as the pcDNA series from Invitrogen, Inc. for mammalian cell expression. "pcDNA-LBD-tag" represents a vector for expression of an epitope-tagged ligand binding domain. To express a receptor immunoglobulin fusion protein, the amplified LBD segment is ligated into an expression vector containing the hinge, CH2 and CH3 domains of an IgG heavy chain as described in Ashkenazi et al, PNAS 88:10535 1991. See e.g., Nature 330, 537-543 (1987) for details relevant to GH receptor.

Figure 1

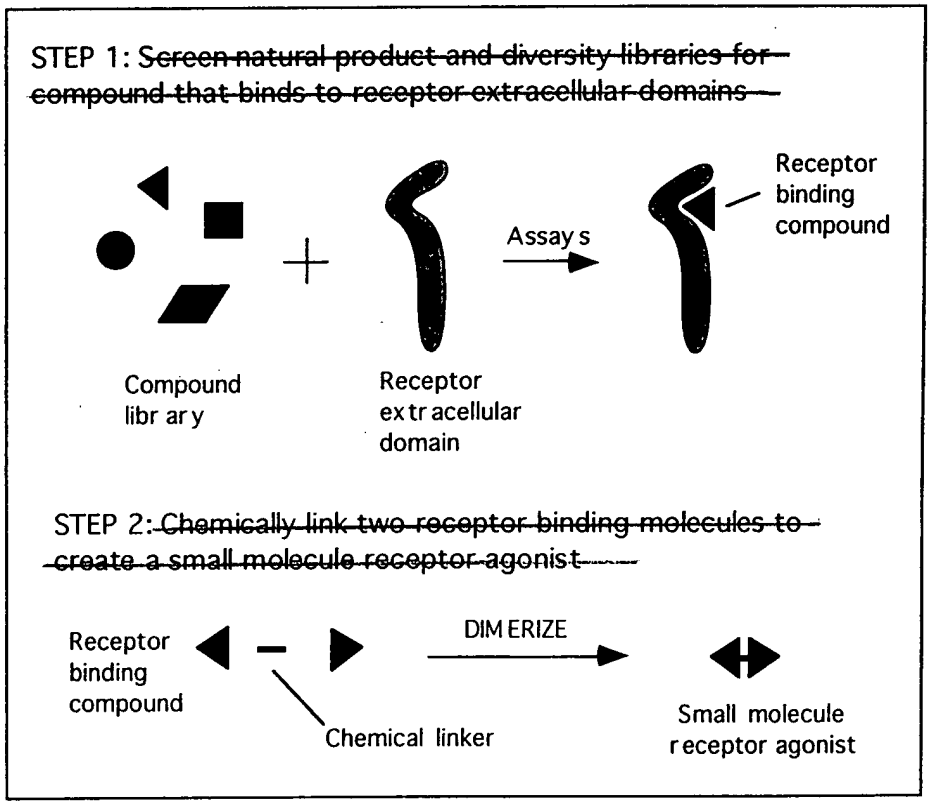

STEP 1: ~~Screen natural product and diversity libraries for compound that binds to receptor extracellular domains~~
Assays
Receptor binding compound
Compound library
Receptor extracellular domain
STEP 2: ~~Chemically link two receptor binding molecules to create a small molecule receptor agonist~~
Receptor binding compound
DIMERIZE
Chemical linker
Small molecule receptor agonist

Fig 2: ~~STIMULATION OF IL-2 PRODUCTION BY A SMALL MOLECULE DIMERIZER~~

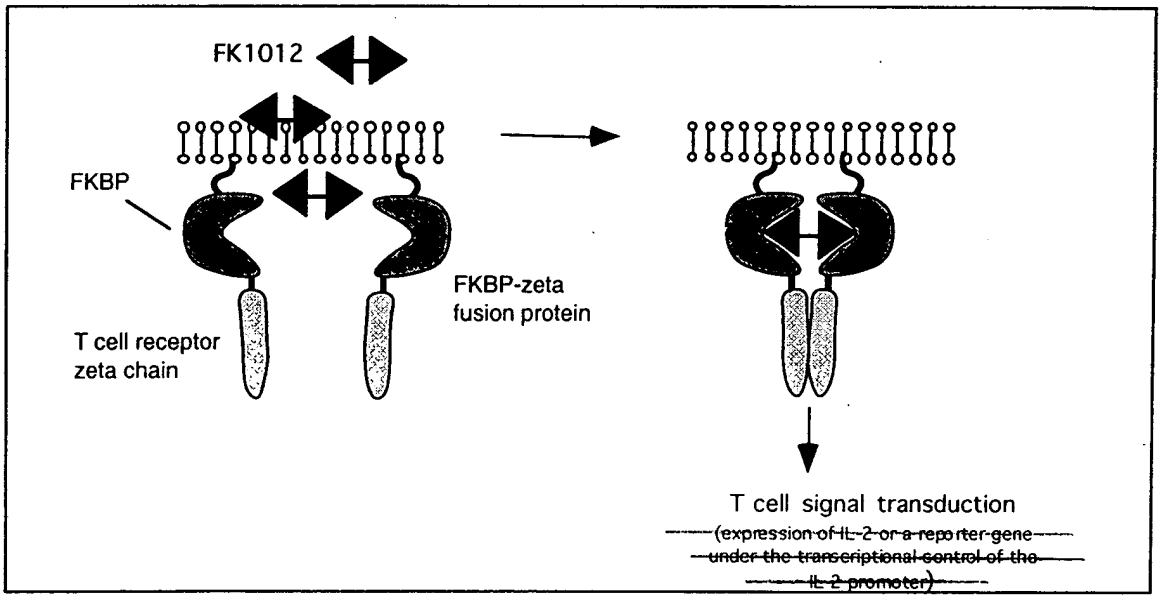


Fig 3: ~~STIMULATION OF ERYTHROPOIETIN RECEPTOR BY A SMALL MOLECULE~~

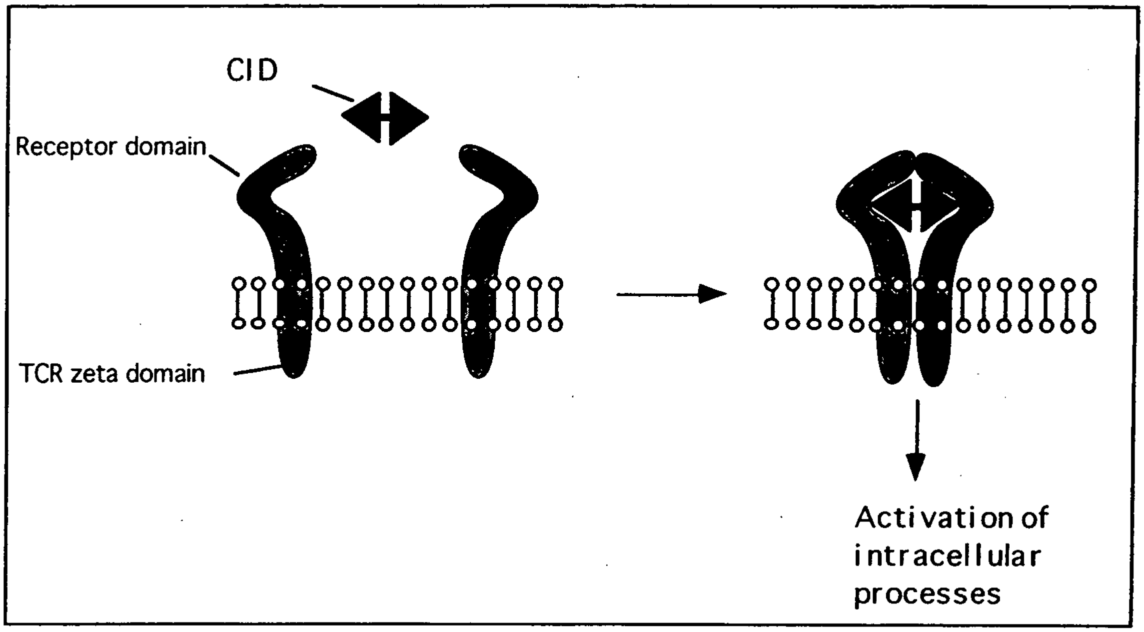


Fig 4: IDENTIFICATION OF REGEPTOR BINDING COMPOUNDS USING A COMPETITIVE LIGAND BINDING ASSAY

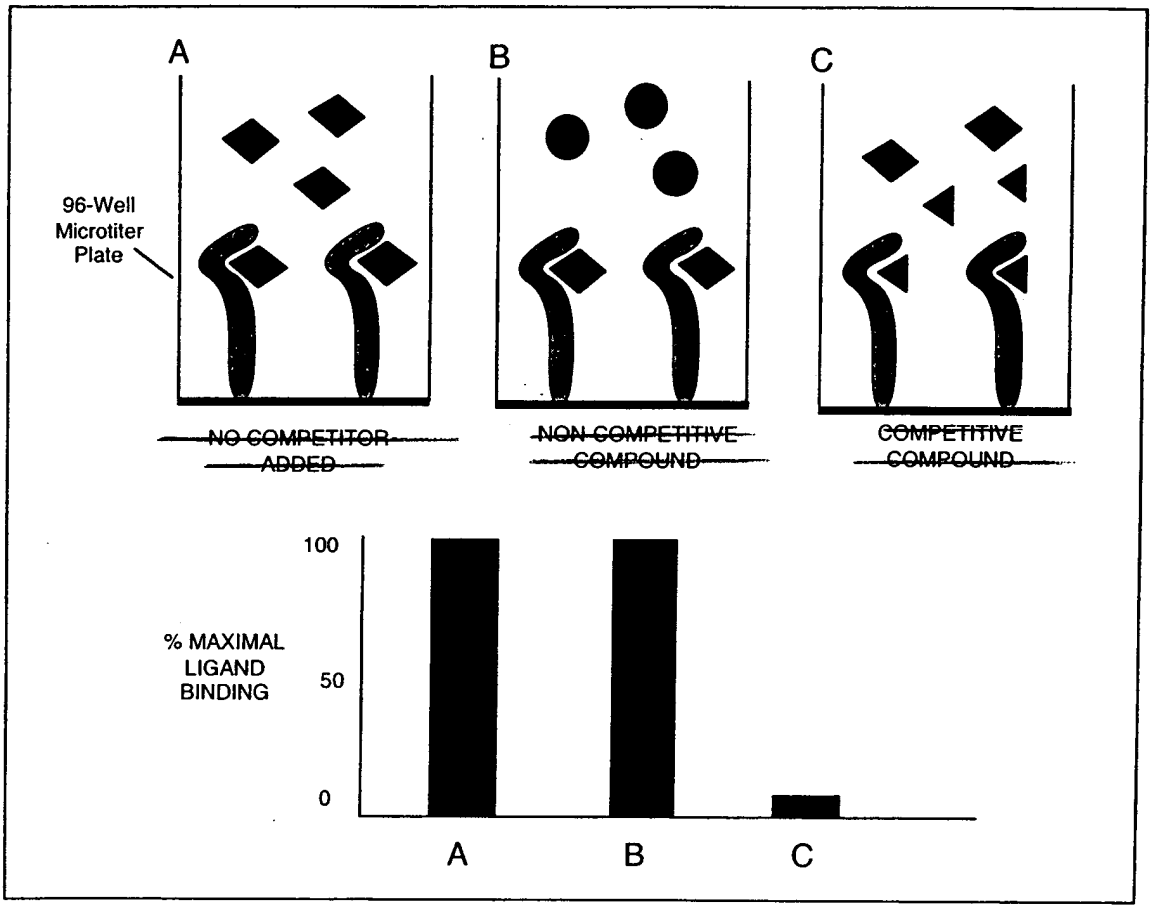


Fig 5: SCREENING MOLECULAR DIVERSITY LIBRARIES FOR COMPOUNDS THAT BIND TO A RECEPTOR EXTRACELLULAR DOMAIN

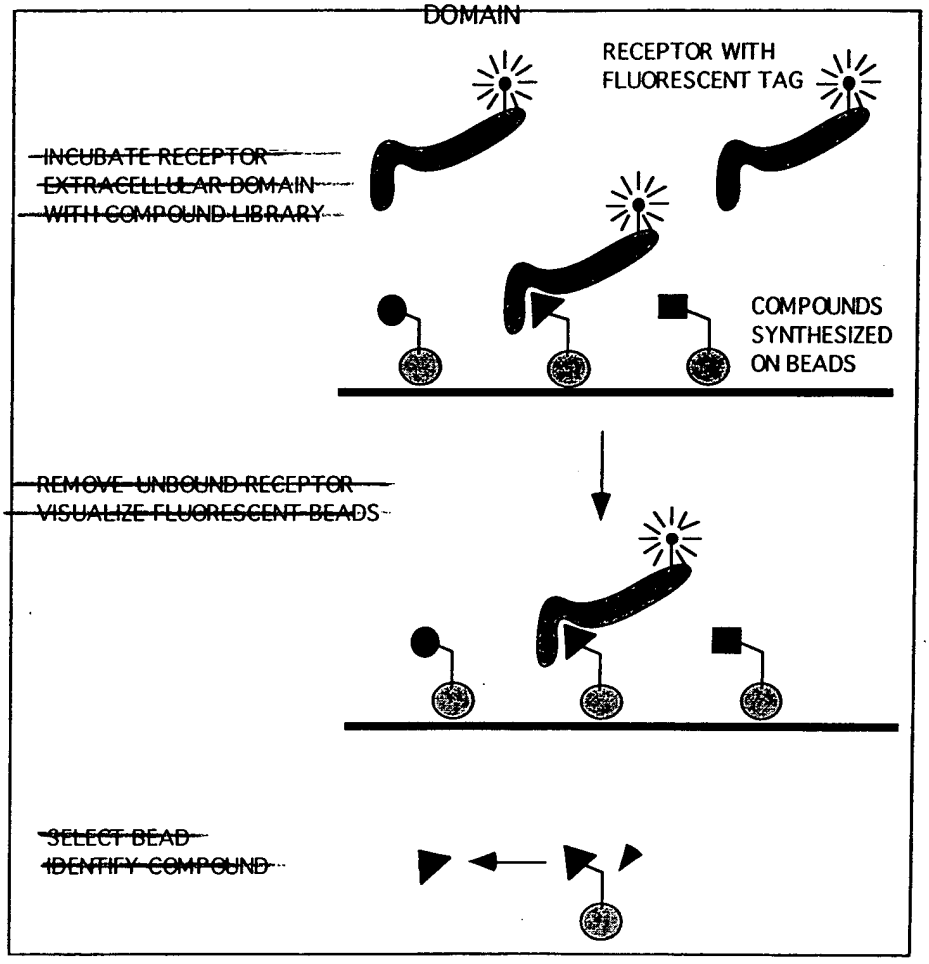
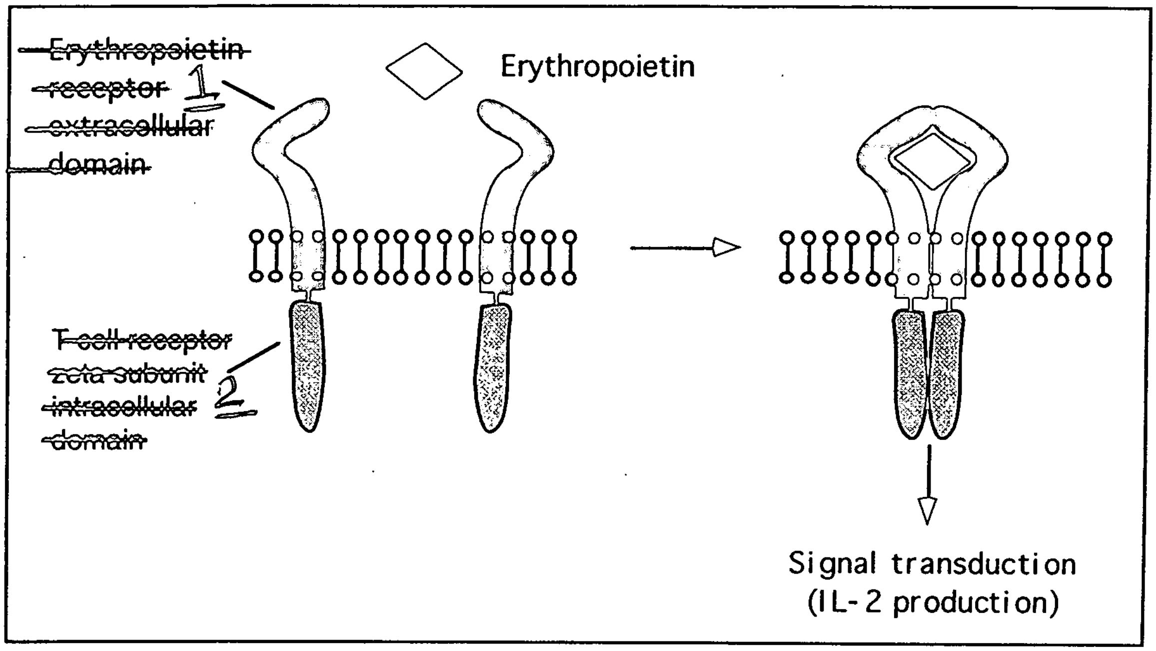


Fig 6: ~~EPO STIMULATES SIGNAL TRANSDUCTION IN AN ENGINEERED CELL LINE~~



~~SMALL MOLECULE BLOCKS EPO RECEPTOR-MEDIATED SIGNAL TRANSDUCTION~~

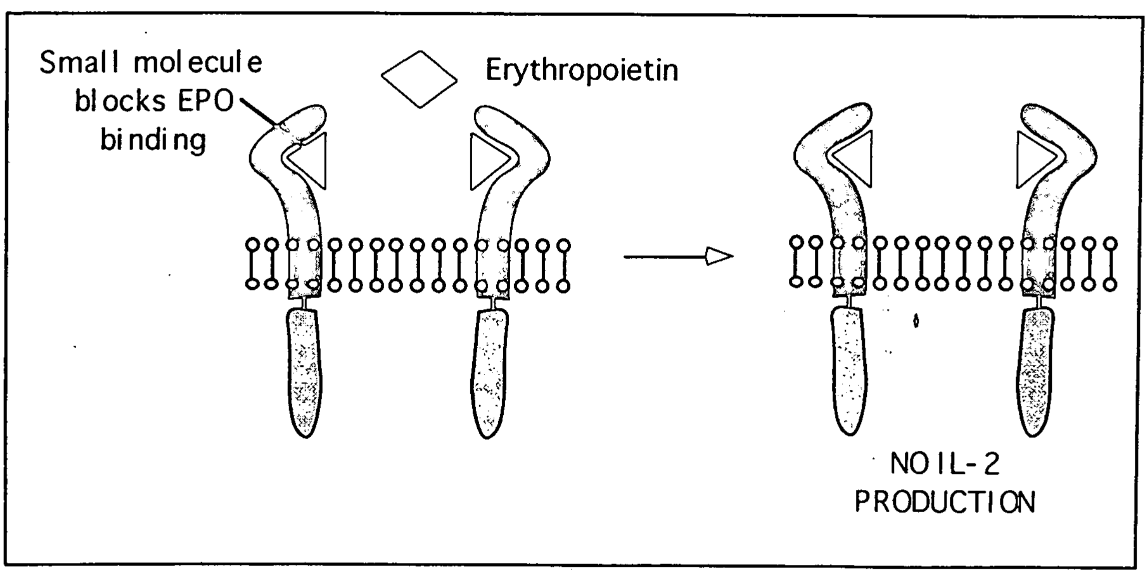
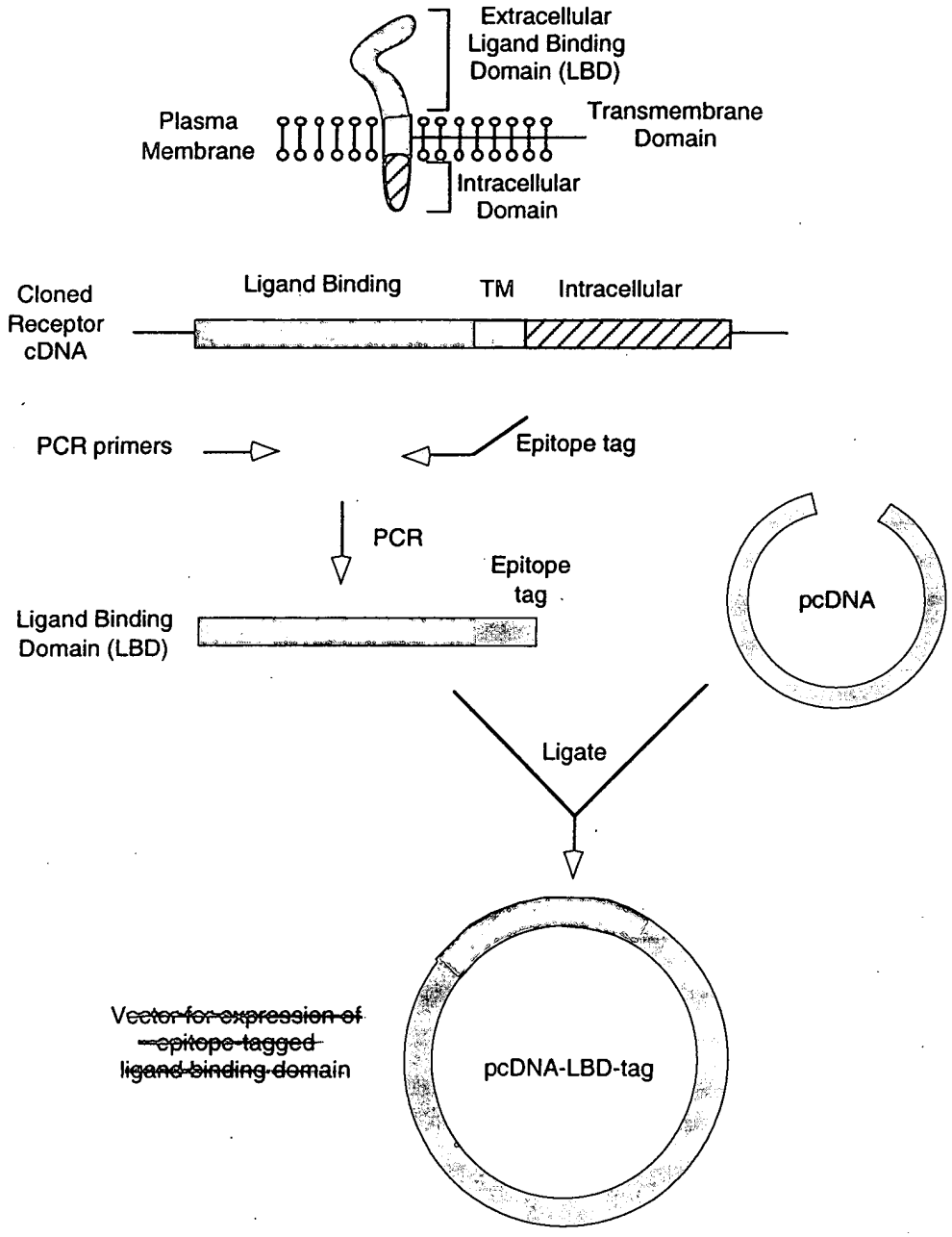


Fig 7: ~~General Method For Construction of Expression Vector for Production of Ligand Binding Domain~~



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